Data Evaluation Record on the Acute Toxicity of Hoe 064619 Technical (Glufosinate-ammonium) to Freshwater Invertebrates - *Daphnia* sp.

EPA MRID Number 48444802

Data Requirement:	EPA DP Barcode	345709
	EPA MRID	48444802
	EPA Guideline	850.1010

Test material: **Hoe 064619 Technical** **Purity:** 98.1%
Common name: Glufosinate-ammonium (degradate)
Chemical name: IUPAC 2-methylphosphinico-acetic acid
CAS name
CAS No.
Synonyms MPA

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental **Date:** 7/8/11

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental, Inc. **Date:** 10/13/11

Primary Reviewer: Catherine Aubee **Signature:**
Biologist, US EPA/OPP/EFED/ERBIV **Date:** 16 March 2012

EPA PC Code 128850

Date Evaluation Completed: 16 March 2012

CITATION: Heusel, R. 1993. 2-Methylphosphinico-acetic acid; substance, technical (Hoe 064619 00 ZC98 0001) - Effect to *Daphnia magna* (Waterflea) in a static-acute toxicity test (method OECD). Unpublished study performed and sponsored by Hoechst AG, Frankfurt am Main, Germany. Study number CE92/071. Study completed April 23, 1993.

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EXECUTIVE SUMMARY:

The study evaluated the acute (48-hour) toxicity of **Hoe 064619 Technical** (98.1% MPA), a transformation product of glufosinate ammonium, to the water flea (*Daphnia magna*) under static conditions. Two tests were conducted due to the acidity of the test material. Daphnids were exposed to nominal concentrations of 0 (negative control), 10, 18, 32, 56, and 100 mg MPA/L in unbuffered test solution. An additional test exposed daphnids to nominal concentrations of 0 (negative control), 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg MPA/L in buffered solution. The pH of the buffered solution was adjusted to 8.0 using sodium hydroxide (NaOH) prior to addition of the daphnids.

Immobility was observed daily. The 48-hour EC_{50} for the unbuffered test solution trial was 37 mg MPA/L, and the 48-hour NOAEC based on immobility was 18 mg MPA/L. Sublethal effects were not observed in the unbuffered solution trial. The 48-hour EC_{50} for the buffered test solution trial was >1000 mg MPA/L, and the 48-hour NOAEC based on immobility was 320 mg MPA/L. Sublethal effects were limited to swimming at the water surface. EFED does not typically use the NOAEC from an acute toxicity test with only minimal replication as a quantitative endpoint, but it may be used for characterization.

Based on the results of this study and the U.S. EPA acute toxicity classification scheme, **Hoe 064619 Technical** in unbuffered solutions would be classified as slightly toxic to daphnids, whereas **Hoe 064619 Technical** in buffered solutions would be classified as practically non-toxic to daphnids.

This study is classified as **acceptable**; it is scientifically sound and satisfies the OCSPP Guideline 850.1010 requirements for an acute toxicity study with freshwater invertebrates exposed to a glufosinate transformation product (MPA).

Results Synopsis

Test Organism Age (e.g., 1st instar): 24 hours
Test Type (Flow-through, Static, Static Renewal): Static

Unbuffered
EC_{50}: 37 mg MPA/L (nominal) 95% C.I.: 32-56 mg MPA/L
Probit Slope: N/A 95% C.I.: N/A

Buffered
EC_{50}: >1000 mg MPA/L (nominal) 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test procedures followed recommended bioassay practices of the Organization for Economic Cooperation and Development (OECD) Guidelines No. 202: Daphnia sp., Acute Immobilization Test and Reproduction Test (April 4, 1984). The study methods and results were assessed according to U.S. EPA OCSPP (formerly OPPTS) 850.1010: *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids* and OECD 202, and differences and/or similarities were described. The reviewer noted following deficiencies and deviations from OCSPP 850.1010:

1. The pH in the unbuffered (unadjusted for pH) test solutions ranged from 4.1 to 8.8, while the pH in the buffered test solutions (adjusted by addition of NaOH) was 8.0. The

pH in the unbuffered solutions was 5.6 in the 56 mg MPA/L test level and was 4.1 in the highest test level, which corresponded with complete immobility. In contrast, pH was 8.0 in every test level in the adjusted solutions, which corresponded with no immobility in treatment levels up to and including 100 mg MPA/L.

2. Analytical verification was not performed on the buffered solutions and was not performed for every test level in the unbuffered solutions. OCSPP guidelines that in static tests, analytical verification should be performed for each test chamber at a minimum at the beginning and end of the test.
3. Neither the LOQ nor LOD were reported.
4. The dilution water was not analyzed for the presence of pesticides, metals, total organic carbon, and particulate matter; OCSPP guidelines have established maximum allowable concentrations for these water quality parameters.
5. Insufficient information was provided on the health and general condition of parental daphnids.
6. A 15-30 minute transition period between light and dark conditions was not employed as suggested by OCSPP guidelines.
7. Water hardness in the unbuffered solutions was 232 mg/L as $CaCO_3$, and in the buffered solutions was 241.4 mg/L as $CaCO_3$; OCSPP guidelines suggest a maximum concentration of 180 mg/L.

The deficiencies and deviations do not have a substantive impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. However, the study author did not report the specific Good Laboratory Practice Standards that were adhered to.

A. MATERIALS:

1. Test material **Hoe 064619 Technical**

Description: White crystalline powder

Lot No./Batch No. : Not reported

Purity: 98.1%

Stability of compound under test conditions: Analytical verification was performed on the test concentrations in the test where the pH was not adjusted. However, only the low, medium, and high test level solutions were analyzed. At time 0, recoveries ranged from 100 to 121% of the nominal test concentrations. Recoveries from the 48-hour solutions ranged from 95 to 113% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of Hoe 064619 Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: *Daphnia magna* Clone 5
(*EPA preferred species is Daphnia magna; OECD preferred species is Daphnia magna or any other suitable Daphnia species*)

Age at test initiation: maximum of 24 hours old (*EPA recommends that Daphnids are in their first instar (≤24 hrs old) and that all organisms are approximately the same size and age; OECD requires age ≤24 hrs old*)

Source: In-house cultures bred in the laboratory
(*EPA requires that all organisms are from the same source. Daphnids from ephippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent*)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was conducted with a control and nominal concentrations of 10, 100, and 1000 mg MPA/L. It is unclear if the solutions in the rangefinder were buffered. No intoxication symptoms were observed. By 48 hours (test termination), there was no immobility in the control or lowest test level. There was 100% immobility present in the 100 and 1000 mg MPA/L test levels.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	24 hours; however, daphnids were raised in laboratory conditions.	*The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Conditions: (same as test or not)	Same temperature and water as testing.	
Feeding:	Daphnids were fed twice weekly an algal suspension (*Scenedesmus subspicatus* from the Collection of Algal Cultures in Germany in filtered water).	
Health: (any mortality observed)	The pre-test health of the parental daphnids was not reported.	
Duration of the test	48 hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test condition		
Static/flow-through	Static	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Type of dilution system for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	None during testing	
Test vessel		
Material: *(glass/stainless steel)* Size: Fill volume:	Glass 300 mL 200 mL	*EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Not reported- deionized water was used to create the M4 medium.	Test water was an artificial mineral medium M4 (Elendt 1990) that was slightly modified. *Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.*

Parameter	Details		Remarks *Criteria*
Water parameters	No pH adjustment	pH adjusted using NaOH	No pH adjustment: Total alkalinity: 44.5 mg/L as $CaCO_3$ Nitrite content: <0.05 mg/L Conductivity: 635-757 µS/cm pH adjusted using NaOH: Total alkalinity: 45.5 mg/L as $CaCO_3$ Nitrite content: <0.05 mg/L Conductivity: 687-1683 µS/cm
Hardness	232 mg/L as $CaCO_3$	241.4 mg/L as $CaCO_3$	
pH	4.1-8.8	8.0	
Dissolved oxygen	8.8-8.9 mg/L	8.7-9.0 mg/L	
Temperature	19.8-20.1°C	19.7-19.9°C	
Total Organic Carbon	Not determined	Not determined	
Particulate matter	Not determined	Not determined	
Metals	Not determined	Not determined	
Pesticides	Not determined	Not determined	
Chlorine	Not determined	Not determined	
			Hardness: *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* *pH:* *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* *Temperature:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) *Dissolved oxygen:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
Number of replicates Negative control: Treatments:	 2 2 per test level		 *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Negative control: Treatments:	 10 10	 *EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing ∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17°C and ≤5 g/L at > 17°C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*
Treatment concentrations Nominal: Measured:	Unadjusted pH: 0 (negative control), 10, 18, 32, 56, and 100 mg MPA/L pH adjusted with NaOH: 0 (negative control), 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg MPA/L Unadjusted pH: LOQ not reported; 11.51, 30.86, and 100.61 mg MPA/L (only low, middle, and high test concentrations were verified) pH adjusted with NaOH: analytical verification not performed	 *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	N/A- no solvent was used	

Parameter	Details	Remarks *Criteria*
		Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.
Lighting	16L:8D Wide spectrum fluorescent lights	*EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period.* *OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	The stability of the test material in the unadjusted test solutions was verified, but only in the low, middle, and high test levels.	
Recovery of chemical Level of Quantitation Level of Detection	Samples from the low, middle, and high unadjusted pH test solutions were analyzed via HPLC with UV detection (216 nm). Not reported Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	- immobility - symptoms of intoxication	

Observation intervals	Daily	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY:

In the unadjusted pH test water, there was no immobility in the control or the 10 and 18 mg MPA/L test levels at 48 hours. In the 32, 56, and 100 mg MPA/L test level, immobility was 25, 100, and 100%, respectively, at 48 hours.

In the test water adjusted with NaOH, there was no immobility in the control or the lowest five test levels throughout the 48 hour test. Immobility was 15, 0, 25, and 55% in the 180, 320, 560, and 1000 mg MPA/L test levels, respectively, at 48 hours.

(*EPA=s Standard Evaluation Procedure (SEP) includes guidance that pretest mortality should be #3% 48 hours prior to testing and control mortality should be #10% at end of study*)

Table 3a: Effect of Hoe 064619 Technical in M4 Medium with Unadjusted pH on Immobility of *Daphnia* sp.

Treatment Nominal (mg MPA/L)	No. of organisms	Observation period			
		Day 1		Day 2	
		No Dead	% mortality	No Dead	% mortality
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	5	25
56	20	8	40	20	100
100	20	20	100	20	100
NOAEC	18 mg/L				
EC_{50}	37 (32-56) mg MPA/L				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A				

Table 3b: Effect of Hoe 064619 Technical in M4 Medium Adjusted with NaOH on Mortality of *Daphnia* sp.

Treatment Nominal (mg MPA/L)	No. of organisms	Observation period			
		Day 1		Day 2	
		No Dead	% mortality	No Dead	% mortality
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	0	0
180	20	0	0	3	15
320	20	0	0	0	0
560	20	0	0	5	25
1000	20	3	15	11	55
NOAEC	32 mg/L				
EC_{50}	911 mg MPA/L				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A				

B. SUB-LETHAL TOXICITY ENDPOINTS:

In the unadjusted pH test water, there were no symptoms of intoxication in the control or any of the test levels at 48 hours.

In the test water adjusted with NaOH, there were no symptoms of intoxication in the control or the lowest three test levels throughout the 48 hour test. Swimming at the water surface was observed for daphnids in the 56 to 1000 mg MPA/L test levels.

Table 4a: Effect of Hoe 064619 Technical in M4 Medium with Unadjusted pH on Symptoms of Intoxication - *Daphnia* sp.

Treatment Nominal (mg MPA/L)	Observation period			
	Day 1		Day 2	
	Symptoms of Intoxication	% affected	Symptoms of Intoxication	% affected
Control	no	0	no	0
10	no	0	no	0
18	no	0	no	0
32	no	0	no	0
56	no	0	no	0
100	no	0	no	0
NOAEC	Not determined			
LOAEC	Not determined			
EC_{50}	N/A			
Positive control, if used % sublethal effect: EC_{50}	N/A			

Table 4b: Effect of Hoe 064619 Technical in M4 Medium Adjusted with NaOH on Symptoms of Intoxication - *Daphnia* sp.

Treatment Nominal (mg MPA/L)	Observation period			
	Day 1		Day 2	
	Symptoms of Intoxication	% affected	Symptoms of Intoxication	% affected
Control	no	0	no	0
10	no	0	no	0
18	no	0	no	0
32	no	0	no	0
56	no	0	yes	not reported
100	no	0	yes	not reported
180	yes	not reported	yes	not reported
320	yes	not reported	yes	not reported
560	yes	not reported	yes	not reported
1000	yes	not reported	yes	not reported
NOAEC	Not determined			
LOAEC	Not determined			
EC_{50}	N/A			
Positive control, if used % sublethal effect: EC_{50}	N/A			

C. REPORTED STATISTICS:

Cumulative immobility data from both the unadjusted and adjusted pH tests were used to determine toxicity values. The EC_{50} value and 95% confidence limits were determined using the computer program designed by Stephan et al., EPA, 1978. The study author selected the test with the narrowest confidence limits (binomial, moving average, or probit methods). Nominal concentrations were used for analysis.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed both datasets via Toxanal 2009 using nominal concentrations. For both datasets, Fisher's Exact test was conducted to determine the NOAEC value. For the unadjusted pH test,

the binomial method was selected because when there are less than two concentrations at which the percent dead is between 0 and 100, neither the moving average nor the probit method can provide statistically sound results. For the test with the solution pH adjusted with NaOH, the data were analyzed via the probit method (goodness of fit probability=0.27).

Test with unadjusted solutions (nominal concentrations)
EC_{50}: 37 mg MPA/L 95% C.I.: 32-56 mg MPA/L
NOAEC: 18 mg MPA/L
Probit Slope: N/A 95% C.I.: N/A

Test with solutions adjusted with NaOH (nominal concentrations)
EC_{50}: >1000 mg MPA/L 95% C.I.: MPAN/A
NOAEC: 320 mg MPA/L
Probit Slope: 2.25 95% C.I.: 1.22-3.27

E. STUDY DEFICIENCIES:

The study author did not analyze the water for particulate matter, pesticides, total organic carbon, metals, or chlorine content.

The study author did not analytically verify the concentration of test material in all test solutions from the unadjusted pH test, and did not analytically verify any of the test concentrations from the test with the adjusted pH solutions. Recovery in the unadjusted solutions was $\geq$ 96% when measured in the low, medium, and high treatment levels, but it is unknown whether pH adjustment may have affected the dissolved fraction of test substance.

F. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement with regard to the test with the unadjusted solutions. However, the reviewer's results for the test with the pH-adjusted solutions differed from the study author's because the reviewer selected the probit method and the study author chose a different test. The reviewer's results are presented in the Executive Summary and Conclusions of this DER.

The pH in the unbuffered (unadjusted for pH) test solutions ranged from 4.1 to 8.8, while the pH in the buffered test solutions (adjusted by addition of NaOH) was 8.0. More specifically, pH in the unbuffered solutions was 5.6 in the 56 mg MPA/L test level and was 4.1 in the highest test level, which corresponded with complete immobility. In contrast, pH was 8.0 in every test level in the adjusted solutions, which corresponded with no immobility in all test levels that were common to the test with the unadjusted solutions. Immobility did not occur until concentrations increased to 180 mg MPA/L. The low pH measured in the test with the unadjusted test solutions likely caused the complete immobility that was observed.

A range-finding study was conducted from June 15 to 17, 1992.

The test performed without pH adjustment was conducted from June 23 to 25, 1992.

A test was conducted using a phosphate buffer from August 26 to 28, 1992. However, there was mortality in the control and all test levels; therefore, these results are not reported

A second test was conducted with a pH adjustment using NaOH, from September 2 to 4, 1992.

G. CONCLUSIONS:

This study is classified as **acceptable**; it is scientifically sound and satisfies the OCSPP Guideline 850.1010 requirements for an acute toxicity study with freshwater invertebrates exposed to a glufosinate transformation product (MPA). The NOAEC and EC_{50} values for the unadjusted test solutions were 18 and 37 mg MPA/L (nominal), respectively. In contrast, the NOAEC and EC_{50} values for the test solutions with pH adjusted using NaOH were 320 and >1000 mg MPA/L (nominal), respectively.

Test with unadjusted solutions
EC_{50}: 37 mg MPA/L 95% C.I.: 32-56 mg MPA/L
NOAEC: 18 mg MPA/L
Probit Slope: N/A 95% C.I.: N/A

Endpoint(s) Affected: immobility
Statistical method: Binomial (reviewer's results)
Endpoints calculated using nominal concentrations.

Test with solutions adjusted with NaOH
EC_{50}: MPA>1000 mg MPA/L 95% C.I.: N/A
NOAEC: 320 mg MPA/L
Probit Slope: 2.25 95% C.I.: 1.22-3.27

Endpoint(s) Affected: immobility and intoxication symptoms limited to swimming at the water surface
Statistical method: Probit method (reviewer's results)
Endpoints calculated using nominal concentrations.

III. REFERENCES:

Organization for Economic Cooperation and Development, 1984. OECD Guideline for Testing of Chemicals. Guideline 202: Daphnia sp., Acute Immobilization Test and Reproduction Test, 04. April 1984.

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Wildlife and Aquatic Organisms, Series 72-2. Acute Toxicity for Freshwater Aquatic Invertebrates.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Test with solutions with unadjusted pH

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	20	0	
1	10	20	0	
2	18	20	0	
3	32	20	5	*
4	56	20	20	*
5	100	20	20	*

Moncie Wright Hoe 064619 Acute test with solutions unadjusted for pH

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	20	100	9.536742E-05
56	20	20	100	9.536742E-05
32	20	5	25	2.069473
18	20	0	0	9.536742E-05
10	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 32 AND 56 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 37.19891

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS

Test with solution pH adjusted using NaOH

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	20	0	
1	10	20	0	
2	18	20	0	
3	32	20	0	
4	56	20	0	
5	100	20	0	
6	180	20	3	
7	320	20	0	
8	560	20	5	*
9	1000	20	11	*

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Moncie Wright  Hoe 064619  Acute test with solution pH adjusted with NaOH
**********************************************************************
CONC.    NUMBER        NUMBER        PERCENT       BINOMIAL
         EXPOSED       DEAD          DEAD          PROB.(PERCENT)
 1000     20            11            55            41.19014
 560      20            5             25            2.069473
 320      20            0             0             9.536742E-05
 180      20            3             15            .1288414
 100      20            0             0             9.536742E-05
 56       20            0             0             9.536742E-05
 32       20            0             0             9.536742E-05
 18       20            0             0             9.536742E-05
 10       20            0             0             9.536742E-05

THE BINOMIAL TEST SHOWS THAT  560  AND +INFINITY CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.


AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 910.7996


RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
   SPAN     G          LC50          95 PERCENT CONFIDENCE LIMITS
 1        1.067224      910.7996      655.1028     +INFINITY


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS             G              H             GOODNESS OF FIT PROBABILITY
    9                   .2087599      1                           .2720655

SLOPE   =               2.246612
95 PERCENT CONFIDENCE LIMITS = 1.22013    AND    3.273095

INTERCEPT=-6.763039

LC50 =    1024.066
95 PERCENT CONFIDENCE LIMITS =  707.1151 AND  2212.269

         LC25 =         512.9771
95 PERCENT CONFIDENCE LIMITS =  361.4371 AND  754.067

         LC10 =         275.3398
95 PERCENT CONFIDENCE LIMITS =  146.1863 AND  386.7982

         LC05 =         189.74
95 PERCENT CONFIDENCE LIMITS =  77.6352 AND  284.1722
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